

# GENETIC CONTROL OF THE UPTAKE OF C<sub>4</sub>-DICARBOXYLIC ACIDS BY *ESCHERICHIA COLI*

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## 1. Introduction

It has long been recognized that micro-organisms catalyse the uptake of many nutrients from their growth media via functionally specialized permeation systems, which may exhibit a high degree of stereo-specificity towards the substrate being transported (for review, see [1]). Although the inducible formation of systems effecting the uptake of some intermediates of the tricarboxylic acid cycles has been demonstrated in pseudomonads [2–4] and in *Aerobacter* [5,6], their nature and specificity remains to be resolved.

It is the purpose of this communication to report that *Escherichia coli* contain a system specifically involved in the uptake of C<sub>4</sub>-dicarboxylic acids. Mutants that lack this system can still metabolize internal C<sub>4</sub>-acids, but fail to grow on these substances as sole carbon sources; this uptake system is specified by a gene (*dct*) located on the *E. coli* genome close to the *xyl* marker.

## 2. Materials and methods

The organisms used in this study are listed in table 1. For experimental use, they were grown aerobically at 37° in a Gallenkamp incubator shaker, in media containing salts [7], necessary amino acids (at 50–100 µg/ml), and the appropriate carbon source at 25 mM. Cultures were harvested at cell densities of 0.3–0.4 mg dry wt/ml; studies of growth on, and oxidation of, various carbon sources were carried out as previously described [7]. Experiments on the uptake of labelled C<sub>4</sub>-dicarboxylic acids were performed at 37° with cells, washed with and suspended in 0.1 M

sodium potassium phosphate, pH 7.4, at 0.068 mg dry wt/ml. Radioactive C<sub>4</sub>-dicarboxylic acids were added at 0.1 mM; at desired time intervals, samples (1 ml) were removed and the cells collected by filtration through Millipore filters (0.45 µ pore size). The filters were washed with 2 ml of buffer and were rapidly transferred to vials, containing 5 ml of Bray's fluid [8], for radioactive assay in a Packard Model 400 scintillation spectrometer. In experiments on competitive inhibition of the uptake of [2,3-<sup>14</sup>C]-fumarate, the unlabelled possible inhibitors were added at 5 mM simultaneously with the labelled C<sub>4</sub>-acid.

Genetic crosses were carried out as previously described [9].

[2,3-<sup>14</sup>C]-fumaric and succinic acids were purchased from The Radiochemical Centre, Amersham. 3-Fluoromalic acid [10] was a gift from Dr. P.W.Kent (Department of Biochemistry, University of Oxford).

## 3. Results and discussion

Wild-type strains of *E. coli*, which grow readily on media containing acetate or C<sub>4</sub>-dicarboxylic acids (such as succinate, fumarate or malate) as sole carbon sources, also readily take up [2,3-<sup>14</sup>C]-fumarate from dilute solution even though they have not been previously exposed to this C<sub>4</sub>-acid (fig. 1). This rapid uptake of labelled fumarate appears to be effected via a transport system which also catalyses the uptake of some other C<sub>4</sub>-dicarboxylic acids; as shown in table 2, the presence of unlabelled succinate, malate or aspartate competes nearly as effectively with the uptake of [2,3-<sup>14</sup>C]-fumarate as does the presence of unlabelled fumarate. In contrast, un-

Table 1  
Organisms used in this study.

| Strain      | Derived from         | Genetic markers                          | Response to streptomycin | Making type    |
|-------------|----------------------|--|--------------------------|----------------|
| AT 2572-FM1 | AT 2572 (a)          | <i>dct</i>                               | S                        | Hfr            |
| AB 2297 (b) | —                    | <i>ilv, pur</i>                          | S                        | Hfr            |
| K2.1t (c)   | —                    | <i>his, arg, thr, leu, pps</i>           | R                        | F <sup>-</sup> |
| K2-1t-FM1   | [AT2572.FM1 × K2-1t] | <i>his, thr, leu, pps, dct</i>           | R                        | F <sup>-</sup> |
| K2-1t-FM2   | [AB2297 × K2.1t-FM1] | <i>his, ilv, pps, dct</i>                | R                        | F <sup>-</sup> |
| K2-FM3      | K2 (c)               | <i>his, trp, arg, thr, leu, xyl, dct</i> | R                        | F <sup>-</sup> |
| P10 (d)     | —                    | <i>thr, leu</i>                          | S                        | Hfr            |

(a) and (b): gifts from Prof. E.A.Adelberg (Yale University Medical School, New Haven).

(c) : see ref. [9].

(d) : gift from Prof. F.Jacob (Institute Pasteur, Paris).

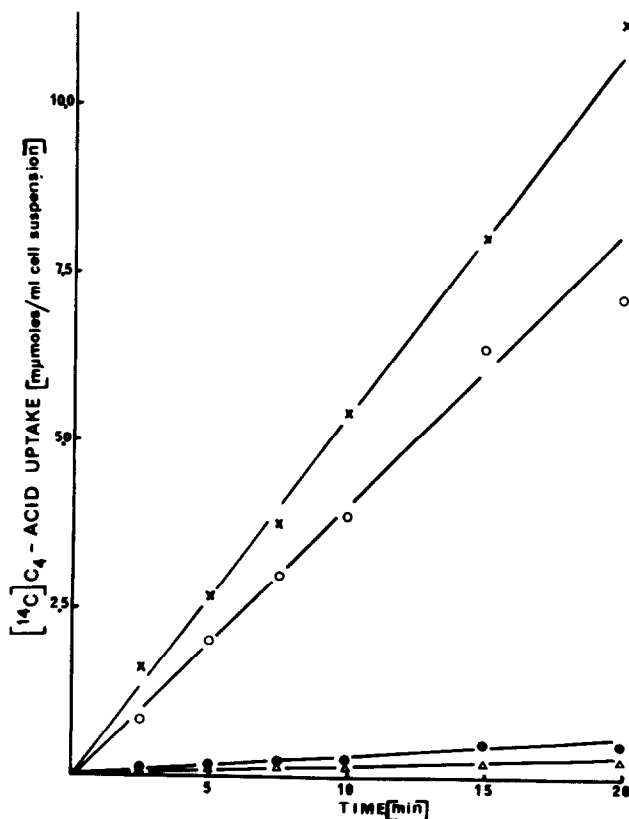


Fig. 1. Uptake of [2,3-<sup>14</sup>C]-fumarate (X,●) and of [2,3-<sup>14</sup>C]-succinate (○,△) by At 2572 and its fluoromalate-resistant mutant FM1 respectively.

Table 2  
Inhibition by C<sub>4</sub>- and C<sub>5</sub>-dicarboxylic acids of [2,3-<sup>14</sup>C]-fumarate uptake into *E. coli*, strain AT 2572.

| [ <sup>12</sup> C] acid added | Inhibition of uptake (%) |
|-------------------------------|--------------------------|
| none                          | 0                        |
| fumarate                      | 96.4                     |
| succinate                     | 93.9                     |
| malate                        | 87.2                     |
| aspartate                     | 70.9                     |
| oxaloacetate                  | 3.3                      |
| glutamate                     | 1.0                      |
| α-oxoglutarate                | 0                        |
| asparagine                    | 0                        |

labelled oxaloacetate exerts a negligible effect on, and neither unlabelled asparagine nor the C<sub>5</sub>-dicarboxylic acids glutamate and α-oxoglutarate compete with, the uptake of labelled fumarate. It follows that the uptake system exhibits specificity towards fumarate > succinate > malate > aspartate ≫ oxaloacetate.

The addition of even small amounts of 3-fluoromalate [10] to cultures of *E. coli* growing on acetate severely inhibits further growth. When suspensions of a variety of wild-type *E. coli* strains were incubated on agar plates containing 25 mM-acetate and 0.05 mM-3-fluoromalate, no growth was visible after 24 hr at 37° but, after 48 hr, small colonies appeared (at a frequency of about 1 per 1 × 10<sup>6</sup> cells plated). These

fluoromalate-resistant mutants differed from their parents in being able to grow in the presence of up to 0.5 mM-fluoromalate but in being unable to grow on malate or fumarate as sole carbon sources, although their rates of growth on acetate, glucose, gluconate, glycerol, pyruvate or lactate were indistinguishable from those of the wild-type organisms. Similarly, the levels of the NAD- and NADP-linked malic enzymes and of malate dehydrogenase were found to be the same in the mutants as in their parents. However, washed suspensions of acetate-grown cultures of these fluoromalate-resistant mutants were no longer capable either of taking up labelled fumarate or labelled succinate rapidly (fig. 1), or of oxidizing fumarate or malate; they oxidized succinate only very slowly. Revertants, which regained the ability to grow on malate, simultaneously recovered all the properties of the wild-type organisms.

The gene specifying this inability to utilize C<sub>4</sub>-dicarboxylic acids was located on the chromosome of *E. coli* in two ways. In the first, AT 2572-FM1, a fluoromalate-resistant mutant of the Hfr strain AT 2572, which transfers its genome in the order *O-his-trp-thr, leu-*, was crossed with the F<sup>-</sup> recipient K2-1t (*his, argHBCE, thr, Leu, pps, str*). All recombinants selected for *his*<sup>+</sup> or *thr*<sup>+</sup> *leu*<sup>+</sup>, by interrupted conjugation, were still able to grow on malate; however, some *arg*<sup>+</sup>-recombinants were unable to utilize malate as growth substrate and had thus presumably acquired the defective allele for dicarboxylic acid transport (*dct*). One such *dct*-recombinant, K2-1t-FM1, was purified and crossed with the Hfr strain AB 2297 (*ilv, pur*), which transfers its genome in the order *O-ilv-argHBCE-thr, leu-*: although many of the recombinants were *ilv*<sup>-</sup>, none was *dct*<sup>+</sup>. The relationship between the positions of the *ilv* and *dct* markers was revealed after interrupted conjugation of K2-1t-FM2, an *ilv, dct*-recombinant from this cross, with the Hfr strain P10 which transfers its genome in the order *O-metA-argHBCE-ilv-str*. As shown in fig. 2a, the ability to grow on malate (*dct*<sup>+</sup>) was introduced about 6 min after the ability to grow in the absence of isoleucine and valine (*ilv*<sup>+</sup>).

A second procedure involved the selection of K2-FM3, a fluoromalate-resistant mutant of the F<sup>-</sup> strain K2 (*dct, his, argHBCE, thr, leu, trp, xyl, str*). Interrupted conjugation with the donor strain P10 (fig. 2B) again showed the *dct* marker to be introduced

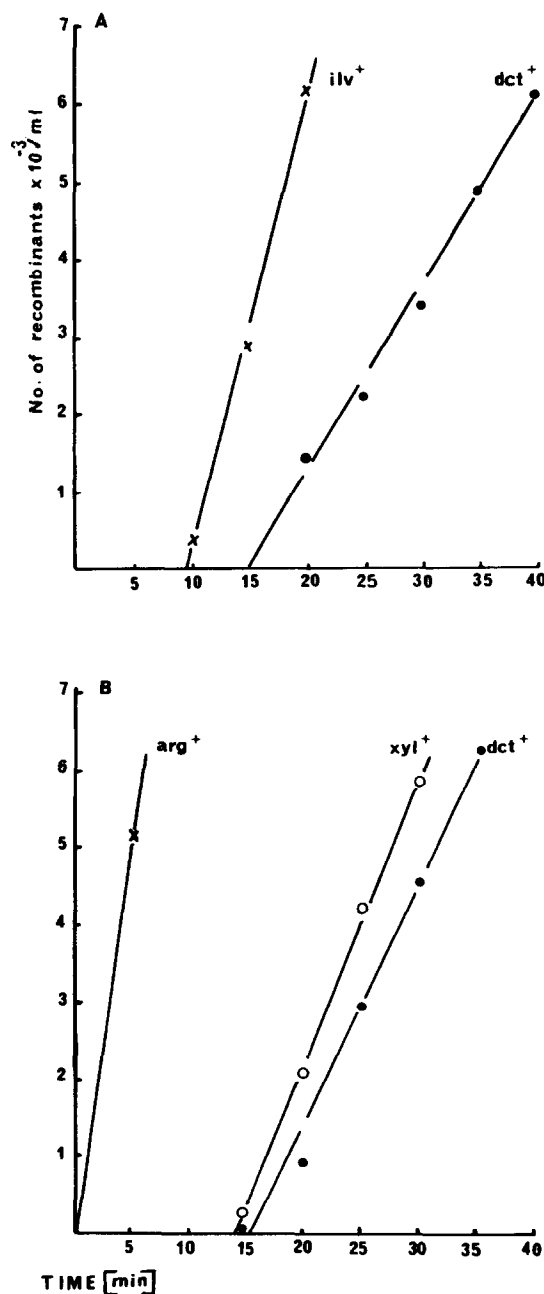


Fig. 2. Kinetics of transfer at 37° of (A) *ilv*<sup>+</sup> and *dct*<sup>+</sup>, and (B) *arg*<sup>+</sup>, *xyl*<sup>+</sup> and *dct*<sup>+</sup>, from P10 to (A) K2.1t.FM2 and (B) K2-FM3.

about 16 min after the onset of genome transfer. Since  $dct^+$  entered 16 min after the introduction of  $arg^+$  and about 1.5 min after  $xyl^+$ , it follows that the gene which specifies the uptake of  $C_4$ -dicarboxylic acids by *E. coli* can be placed at about 70 min on the linkage map described by Taylor and Trotter [11], the order being  $...ilv...xyl...dct...$ .

Although *dct*-mutants of *E. coli* are markedly impaired in their ability to take up labelled succinate from dilute solutions, to oxidise succinate, or to grow on succinate, they are not totally devoid of the ability to utilize exogenous succinate. This suggests that some mechanism other than that specified by the *dct*-gene can effect the transport of succinate into the cell, albeit at a low rate. The nature of this system will be described in a subsequent publication.

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